Characterization of Neutral Fragments in Tandem Mass Spectrometry: A Unique Route to Mechanistic and Structural Information

Michael J. Polce, Šárka Beranová, Michael J. Nold and Chrys Wesdemiotis

Department of Chemistry, University of Akron, Akron, Ohio 44325-3601, USA

The neutral species eliminated upon fragmentation of fast-moving mass-selected ions can be directly identified by collisional ionization and detection in neutral fragment reionization mass spectra. Establishment of the (NfR) identity of neutral fragments yields valuable insight into the decomposition mechanism of a precursor ion, as demonstrated for fullerene and alkali metal iodide cluster ions as well as metal ion adducts of amino acids. In addition, neutral fragment reionization also provides structural information that may not be available from the complementary ionic fragments alone; this is illustrated in the differentiation of isomeric mononucleotides. The parameters influencing the appearance of NfR spectra are discussed and the scope and general applicability of the method are briefly evaluated.

KEYWORDS: tandem mass spectrometry; neutral fragment reionization; fullerenes; cluster ions; nucleotides; metalated amino acids

INTRODUCTION

Tandem mass spectrometry (MS/MS) is widely used both in the analysis of molecular structures and in fundamental gas-phase ion chemistry studies. In most cases, structural information in MS/MS experiments is derived from the unimolecular reactions of a mass-selected precursor ion, most commonly induced via collisionally activated dissociation (CAD). Although such decompositions produce simultaneously ionic and neutral fragments (Scheme 1), only the charged particles can be identified in conventional MS/MS. The co-produced neutrals and, thus, any mechanistic or structural insight residing in them generally remain accessible. This deficiency is alleviated by neutral fragment reionization (NfR), a variant of neutralization-reionization mass spectrometry (NRMS). In NfR, the neutral species released upon CAD of fast (keV) moving ions are collisionally ionized to become directly analyzable. The ions emerging upon collisional ionization are separated by their mass-to-charge ratios and recorded as neutral fragment reionization mass spectra. Establishment of the identity of neutral fragments yields valuable insight into the decomposition mechanism of a precursor ion, as demonstrated for fullerene and alkali metal iodide cluster ions as well as metal ion adducts of amino acids. In addition, neutral fragment reionization also provides structural information that may not be available from the complementary ionic fragments alone; this is illustrated in the differentiation of isomeric mononucleotides. The parameters influencing the appearance of NfR spectra are discussed and the scope and general applicability of the method are briefly evaluated.

The prototype NfR experiment was reported by McLafferty et al., who detected the neutral fragments from collisionally activated and metastable acetone cations. Soon thereafter, the groups of Holmes and Terlouw recognized the promise of the method and demonstrated its usefulness by identifying the neutral fragments from metastable ions with puzzling decomposition mechanisms; the process of ionizing such metastably generated neutrals was specifically named collisionally induced dissociative ionization (CIDI), Table 1.

The first problem solved by CIDI was the nature of the neutral (C, H, N) fragment cleaved from metastable aniline ions, which was shown by Burgers et al. to be HNC, not the thermodynamically more stable HCN. Later CIDI studies on metastable methyl acetate cations by the same researchers provided strong evidence that CH₃C(=O)OCH₃⁺ cogenerates a substantial amount of 'CH₃OH radicals, together with the expected 'CH₃, during fragmentation to yield CH₃CO⁺. Similarly, the McLafferty rearrangement of metastable n-hexanoic acid ion to C₆H₅⁺ was revealed by van Baar et al. to release CH₃COOH, not the enol...
Neutral fragment reionization. In NR, collisional activation dissociation (CAD) is utilized to examine, based on the principle of microscopic reversibility, for learning how larger ions fragment, which in turn promises the advantage of directly seeing neutral dissociation products. Knowledge of their identity is essential.

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Few NR studies have so far explored the implications for molecular structure elucidation of the structural information residing in neutral CAD fragments. Wu and Fenselau23 and Cordero and co-workers3,16b reionized peptides cleaved from proton-bound dimers and found that collisional ionization produces sequence indicative N-terminal ions. Additionally, Squire et al.16c showed that NR distinguishes leucine from isoleucine residues when they are eliminated from a protonated peptide’s C-terminus. Here, we also discuss how NR can help characterize protonated nucleotide isomers.24

**NEUTRAL FRAGMENT REIONIZATION**

NR involves two sequential collisions. The first collision promotes CAD, giving rise to ionic and neutral fragments (Scheme 2). After removal of the ionic fragments and any unaffected precursor ions, the neutral fragments are subsequently ionized in the second collision. This procedure is very similar to that encountered in neutralization–reionization (NR)4–6 where the first collision neutralizes the precursor ion and the second reionizes the intermediate neutral (after deflection of non-neutralized ions), viz. Scheme 2. Whether the first collision causes CAD or charge exchange depends, inter alia, on the target (see below). Helium has been the preferred CAD gas in NR studies, because its high ionization energy and negative electron affinity25 minimize the degree of concomitant charge exchange.26 In contrast, metallic targets,26 xenon,26 dimethyl disulfide27 and trimethylamine18,28,29 have been found primarily to effect neutralization (i.e. electron transfer) and should be avoided in NR measurements.

**Scheme 2.**
Regarding the second, reionizing collision, \( \text{O}_2 \) is the most efficient collision gas for the production of cations, causing the least fragmentation.\(^{30}\) We have found trimethylamine (TMA) or Xe to be adequate choices for generating anions.\(^{19,31}\) The collisional ionization yields are \( \approx 2\% \) for cations and \( \approx 0.1\% \) for anions. The charges of the precursor ion and of the ultimate reionization products may be indicated by superscripts to the \( \text{N}_f\text{R} \) acronym. For example, the \( ^{+}\text{N}_f\text{R}^- \text{He/Xe} \) spectrum of \( (\text{CsI})_2\text{Cs}^+ \) is acquired by dissociating \( (\text{CsI})_2\text{Cs}^+ \) cations (with He) and subsequently ionizing the collisionally generated neutral fragments to anions (with Xe).

\( \text{N}_f\text{R} \) in competition with NR

As mentioned above and illustrated in Scheme 2, collision of a mass-selected precursor ion with a gaseous target can cause CAD and/or charge exchange; both of these processes create neutral species. In addition, neutral fragments may be formed by the spontaneous decomposition of remaining metastable ions (i.e. of precursor ions that did not collide but still possess sufficient internal energy to undergo a unimolecular reaction). In the Akron tandem mass spectrometer (see Experimental), collision-induced fragmentation at gas pressures corresponding to \( \geq 20\% \) beam attenuation generally yields \( >20\% \) more fragments than does metastable ion fragmentation. Consequently, in the presence of a collision gas, the proportion of metastably generated neutral fragments is negligible compared with those arising by CAD.

In an \( \text{N}_f\text{R} \) experiment, the extent of concomitant NR can be minimized by proper target choice (see above); however, it may be impossible to eliminate it completely. Alternatively, some precursor ions may rather dissociate than undergo charge exchange, even with superior neutralization targets. The relative cross-section for neutralization vs. dissociation depends on the nature of the precursor ion, the thermochemistry and Franck–Condon factor of charge exchange,\(^{26,27}\) as well as on other, not completely understood variables. Which product ions in the ultimately observed spectrum originate from neutral fragment reionization (i.e. from dissociative collisions) and which from neutralization–reionization (i.e. from neutralizing collisions) can nevertheless be assessed by contrasting spectra taken with He (which primarily dissociates) vs. trimethylamine (TMA) or Xe (which primarily neutralize). This is illustrated here for two precursor ions that exhibit different behavior.

Figure 1 compares the \( ^{+}\text{N}_f\text{R}^+ \text{He/O}_2 \) and \( ^{+}\text{NR}^+ \text{TMA/O}_2 \) spectra of ionized glycine.\(^{32}\) The major CAD process of this radical cation \( \geq 70\% \) of total fragments, Fig. 2(a)] is the decomposition \( ^{+}\text{H}_2\text{NCH}_2\text{COOH} \rightarrow ^{+}\text{H}_2\text{N}=\text{CH}_2 \text{ (m/z 30) + COOH (45 u)} \), which eliminates the carboxyl radical. Since only one major neutral fragment is released, the \( ^{+}\text{N}_f\text{R}^+ \) spectrum [Fig. 1(a)] basically represents the CIDI spectrum of 'COOH. Confirmatory evidence that Fig. 1(a) contains reionized 'COOH is provided by the similarity of the fragmentation patterns in this figure and the CAD spectrum of authentic 'COOH [Fig. 2(b)].

![Figure 1](image1)

**Figure 1.** (a) \( ^{+}\text{N}_f\text{R}^+ \text{He/O}_2 \) and (b) \( ^{+}\text{NR}^+ \text{TMA/O}_2 \) spectra of ionized glycine, \( ^{+}\text{H}_2\text{NCH}_2\text{COOH (m/z 75)} \), formed by El. TMA = trimethylamine. Part (a) essentially represents the CIDI spectrum of 'COOH (see text).

Finally, it is pointed out that the \( ^{+}\text{N}_f\text{R}^+ \) spectrum of \( ^{+}\text{H}_2\text{NCH}_2\text{COOH (Fig. 1(a)] also includes a minor peak at m/z 30 that cannot be formed from reionized COOH. This ion presumably originates from the minor CAD channel \( ^{+}\text{H}_2\text{NCH}_2\text{COOH} \rightarrow ^{+}\text{COOH (m/z 45 in Fig. 2(a)]}, which liberates the radical \( \text{H}_2\text{NCH}_2^\cdot \) (30 u).

The \( ^{+}\text{NR}^+ \text{TMA/O}_2 \) spectrum of ionized glycine [Fig. 1(b)] duplicates all features of the \( ^{+}\text{N}_f\text{R}^+ \) spectrum, indicating that a substantial portion of the precursor ion undergoes CAD, even with TMA. In addition, a recovered molecular ion (survivor ion or

![Figure 2](image2)

**Figure 2.** CAD spectra of (a) ionized glycine, \( ^{+}\text{H}_2\text{NCH}_2\text{COOH (m/z 75)} \) and (b) the carboxyl cation, \( ^{+}\text{COOH (m/z 45)} \), formed by El of formic acid. \( \text{O}_2 \) served as the collision gas.
recovery peak at \( m/z 75 \) and additional fragments (e.g. \( m/z 74, 57 \) and 30) appear and can readily be explained as arising from the fraction of glycine ions that were neutralized–reionized; overall, CAD prevails with either target. For this reason, the \( ^+\text{NR}^+ \) spectrum of ionized glycine is not an adequate reference spectrum of neutral glycine (see below).

The situation changes with \( \text{C}_{60}^+ \) precursor ions.\(^ {19} \) Now, the \( ^+\text{NR}^+ N_2/O_2 \) and \( ^+\text{NR}^+ \) TMA/O\(_2\) spectra differ dramatically, cf. Fig. 3 (in this case, \( N_2 \) was used for CAD because He can also be entrapped in the fullerene cage).\(^ {33} \) The large survivor ion in the \( ^+\text{NR}^+ \) spectrum [Fig. 3(b)] can only result from neutralization. Moreover, the fragment ion pattern in this spectrum closely resembles that in the CAD spectrum of \( \text{C}_{60}^+ \) [Fig. 3(c)],\(^ {19} \) indicating that they are formed from dissociations of the \( \text{C}_{60}^+ \) survivor ion. The small relative abundance of recovered \( \text{C}_{60}^+ \) in the \( ^+\text{NR}^+ \) spectrum [Fig. 3(a)] reveals, on the other hand, that \( N_2 \) targets effect little neutralization and that the abundant ion group extending up to \( \sim \text{C}_{28}^+ \) must mainly originate from the neutral fragments eliminated from \( \text{C}_{60}^+ \) by CAD (see below for interpretation). In this case, changing the target has a dramatic effect on the competition between CAD and neutralization.

Mixtures of neutral fragments

As mentioned above, CAD gives rise to a mixture of neutral fragments, all of which are reionized concurrently (see \( \text{N}_2 \text{R} \) entry in Table 1). The interpretation of \( \text{N}_2 \text{R} \) spectra is, therefore, facilitated if reference reionization (CIDI) spectra of individual neutral fragments are obtained independently. This can be achieved by finding precursor ions that dissociate to yield just the desired neutral fragment. Specific neutrals can be generated by dissociation of metastable ions,\(^ {5,6,8–16} \) however, the flux of metastably generated neutral fragments is not large enough in our instrument to afford usable CIDI spectra. All reference CIDI spectra from our laboratory have been measured on CAD-generated neutral losses, from precursors whose CAD liberates mainly one neutral species. The latter condition is fulfilled by several classes of ions,\(^ {3,11,16,23,35–38} \) especially amine radical cations,\(^ {35,36} \) and proton-bound dimers,\(^ {3,11,16,23} \) which can be used to prepare individual radicals and molecules, respectively. For example, we showed above that CAD of ionized glycine (i.e. an amine radical ion) supplies \( \text{COOH} \) radicals of adequate purity [see Fig. 1(a) for the CIDI spectrum of \( \text{COOH} \)]. Proton-bound dimers can be used analogously, as exemplified in Fig. 4, which shows the CIDI spectra of glycine (Gly) and alanine (Ala) molecules formed from the dimers Gly–H\( ^+ \)–Gly and Ala–H\( ^+ \)–Ala, respectively. The ions present in these CIDI spectra also appear in the electron impact (EI) spectra of Gly and Ala (insets) and the CAD spectra of ionized Gly [Fig. 2(a)] and Ala (not shown), as expected. Note, however, that the relative peak intensities in EI, CAD and CIDI spectra vary, probably because of the different internal energies deposited in these processes.\(^ {40} \)

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**Figure 3.** (a) \( ^+\text{NR}^+ N_2/O_2 \) spectrum of \( \text{C}_{60}^+ \). The region up to \( m/z \approx 400 \) in this spectrum arises from the neutral fragments eliminated from \( \text{C}_{60}^+ \) upon CAD with \( N_2 \). The ions above \( m/z \) 400 are due to the small fraction of \( \text{C}_{60}^+ \) that underwent neutralization, instead of CAD, with the \( N_2 \) targets (see text). (b) \( ^+\text{NR}^+ \) TMA/O\(_2\) spectrum of \( \text{C}_{60}^+ \). (c) CAD spectrum of \( \text{C}_{60}^+ \) using \( O_2 \) as the collision gas. This spectrum contains the ionic fragments generated from \( \text{C}_{60}^+ \). \( \text{C}_{60}^+ \) was formed by EI in all spectra. Reprinted from Ref. 19.

**Figure 4.** CIDI mass spectra of (a) 4.0 keV Gly and (b) 4.0 keV Ala, eliminated from the proton-bound dimers Gly–H\( ^+ \)–Gly and Ala–H\( ^+ \)–Ala, respectively (formed by FAB). The amino acids were liberated by CAD with He and reionized by collisions with \( O_2 \). The insets show EI spectra of Gly and Ala. Reprinted from Ref. 16a.
An alternative route to reference collisional ionization spectra of an individual neutral species is by neutralization–reionization (NR) of the corresponding ion.\(^3,17\) When using this approach, care should be taken that the NR spectrum is not contaminated by \(N_xR_x^+\) as was the case for ionized glycine [cf. Figs 1 and 4(a)]. Further, the neutral accessed in the neutralization step must not decompose before reionization otherwise the NR spectrum represents a neutral mixture, not the desired species alone. NR can provide reference spectra for stable molecules, such as alkenes\(^1,7\) or enols,\(^4,32\) but is not suitable for weakly bonded species. For example, the \(\text{NR}^+\) TMA/O\(_2\) spectrum of \(^+\text{COOH}\) (Fig. 5) is a poor reference spectrum of \(^+\text{COOH}\), because, as documented by Holmes et al.,\(^1,1\) the neutralization step deposits enough energy in the incipient radical to cause extensive dissociation to \(\text{CO}_2^+ + \text{H} + \text{CO} + \text{OH}\) before reionization (Fig. 5). Fortunately, \(^+\text{COOH}\) can be generated by a different pathway, namely via CAD of the glycine ion, as outlined above [Fig. 1(a)]. If the latter choice is not available, the EI spectrum of a neutral or the CAD spectrum of its ionized form (or even similar data for a chemically related species)\(^1\) may provide some information on what types of product ions are to be expected upon collisional ionization of this neutral.

The availability of reference CIDI spectra, it is virtually impossible to identify quantitatively every neutral fragment generated from a given precursor ion. The power of the \(N_xR_x^+\) method rather lies in the qualitative detection of certain species in neutral CAD mixtures to thereby decipher puzzling mechanistic (or structural) problems. For compositional assignments of these mixtures, it is important to recognize the following limitations.

Equivalent amounts of different neutral fragments may not contribute similarly to the \(N_xR_x^+\) spectrum. The larger neutral species, which have higher kinetic energies and hence superior transmission and collection efficiencies,\(^2,42\) usually dominate the ultimately observed spectra.\(^1\) A higher translational energy also appears to favor the larger fragments, but it also increases the internal energy deposition associated with the target used. Owing to this complexity, it is virtually impossible to identify quantitatively every neutral fragment generated from a given precursor ion. The power of the \(N_xR_x^+\) method rather lies in the qualitative detection of certain species in neutral CAD mixtures to thereby decipher puzzling mechanistic (or structural) problems. For compositional assignments of these mixtures, it is important to recognize the following limitations.

Several variables affect the contribution of a specific neutral to an \(N_xR_x^+\) spectrum, including the kinetic and internal energy of the species, its structure and the reionization efficiency and internal energy deposition associated with the target used. Owing to this complexity, it is virtually impossible to identify quantitatively every neutral fragment generated from a given precursor ion. The power of the \(N_xR_x^+\) method rather lies in the qualitative detection of certain species in neutral CAD mixtures to thereby decipher puzzling mechanistic (or structural) problems. For compositional assignments of these mixtures, it is important to recognize the following limitations.

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![Figure 5](image-url)  
*Figure 5. \(\text{NR}^+\) TMA/O\(_2\) spectrum of \(^+\text{COOH}\) (m/z 45), formed by EI of formic acid. It resembles qualitatively the \(\text{NR}^+\) Xe/He spectrum of \(^+\text{COOH}\) published by Holmes et al.\(^1,1\)*

![Figure 6](image-url)  
*Figure 6. \(\text{NR}^+\) He/O\(_2\) spectra of (a) [Ala–Gly]\(^+\) and (b) [Gly–Ala]\(^+\), formed by FAB at 8.0 keV. Gly and Ala cleaved from these peptide ions have kinetic energies of 4.1 and 4.8 keV, respectively. Reprinted from Ref. 16a.*

**Scheme 3.**
reverse activation energy could produce internally excited neutral fragments which, according to studies by Harnish and Holmes, have a reduced reionization cross-section; this will in turn decrease their contribution to an 
$N_f R$ spectrum. Consideration of these problems is essential for the correct interpretation of 
$N_f R$ data.

**EXPERIMENTAL**

$N_f R$ involves high-energy collisions. The experiments described in this study were conducted on a modified VG AutoSpec E$_1$BE$_2$ tandem mass spectrometer, housing two collision cells and an intermediate deflector in the field-free region between E$_1$B (MS-1) and E$_2$ (MS-2). Precursor ions were formed by EI at 70 eV or by fast atom bombardment (FAB) with a Cs$^+$ ion gun operated at ~20 keV. They were accelerated to 8 keV, mass selected by MS-1 and subjected to CAD with He or N$_2$ in the collision cell following the magnet. All ions exiting this cell were removed by electrostatic deflection and the remaining beam of neutral fragments was reionized in the collision cell preceding E$_2$. The newly formed ions were mass analyzed by MS-2 and recorded as the respective N$_f R$ spectrum. NR spectra were acquired similarly by replacing the first collision gas with trimethylamine or Xe.

All samples used are commercially available and were introduced into the mass spectrometer as received from the manufacturer. The spectra shown are multi-scan summations and their relative abundances are reproducible to within ±15%. Absolute abundances (i.e. N$_f R$ yields) were calculated by dividing the abundance of a specific ion or of the total ions exiting the reionization cell by the abundance of the unattenuated precursor ion subjected to CAD. Reionization yields were calculated by dividing the total ion current emerging from reionization by the flux of the neutral fragments (assumed to be identical with the total fragment ion current in the corresponding CAD spectrum). Total N$_f R$ and reionization yields range between ~10$^{-6}$ and 10$^{-4}$ and between ~10$^{-3}$ and 10$^{-2}$, respectively (±50%).

The pressure of each collision gas was adjusted to achieve 20% (50% for C$_{2n}$) reduction of the precursor ion abundance. Glycero, thiglycerol, a 5:1 mixture of dithiothreitol and dithioerythritol and a 1:1 mixture of thioglycerol and 2-hydroxyethyl disulfide served as matrices for FAB ionization. To protonate the sample, it was acidified with hydrochloric or trifluoroacetic acid.

To form metal ion adducts, the sample was mixed with a solution of a suitable metal salt in one of the aforementioned matrices. The alkali metal iodide clusters were ionized by FAB without any matrix. Buckminsterfullerene was thermally desorbed at 375°C from the direct insertion probe.

**C$_{2n}$ LOSSES FROM C$_{60}^+$ FULLERENE CATIONS**

Singly and multiply charged fullerene cations that have been supplied with sufficient internal energy for dissociation (e.g., upon EI or CAD) primarily undergo nominal C$_{2n}$ eliminations (Scheme 4). These reactions could involve n sequential C$_2$ cleavages or the loss of larger, intact C$_{2n}$ units. Appearance energy measurements cannot help determine the true mechanism owing to the large kinetic shifts associated with C$_{2n}$ decompositions. The problem can, however, be reliably solved by neutral fragment reionization, which ascertains directly the identity of the neutral fragments.

CAD of C$_{60}$ gives rise to an abundant fragment ion series that ranges between C$_{52}$ and C$_{58}$ [Fig. 3(c)] and formally arises by the above-mentioned C$_{2n}$ losses. Another group of fragment ions stretches from C$_7^+$ to C$_{28}$ and results, based on studies by Doyle and Ross and us, from consecutive catastrophic dissociations of [C$_{60}$ − C$_{2n}$]$^+$, which release very small neutral C$_{2n}$.

The neutral CAD fragments from C$_{60}$ lead to the N$_f R^+$ N$_2/O_2$ spectrum in Fig. 3(a), which contains abundant ions extending up to C$_{28}$. (The higher mass ions arise, at least in part, from the competitive NR process.) Clearly, CAD of C$_{60}$ with N$_2$ does liberate large neutral moieties, which must originate from the primary C$_{2n}$ cleavages (see above). The dominant product ions from reionization of C$_{2n}$ are odd carbon clusters (C$_{11}$, C$_{15}$, C$_{19}$), similar to the magic clusters found in the laser ablation of graphite. This can be explained as follows: (1) collisional ionization deposits high average internal energies, resulting in extensive fragmentation of the reionized C$_{2n}$, (2) The C$_{2n}$ mixture consists of small, even-numbered carbon clusters (2n < 30). Such clusters are known to dissociate preferably by C$_1$ and C$_{2n}$ losses, which would produce odd cluster ions from the even-numbered C$_{2n}$, as indeed is observed.

The negligible abundance of C$_2^+$ in the N$_f R^+$ spectrum in Fig. 3(a) does not necessarily rule out the possibility of sequential C$_2$ losses. C$_2$ from 8 keV C$_{60}$ has only 267 eV of kinetic energy and would therefore be subject to serious transmission and scattering losses (see above). In addition, its reionization efficiency can be expected to be low. In our instrument, mass discrimination effects become severe when the translational energy drops well below 1 keV. To minimize these problems, multiply charged C$_{60}^+$ (z = 2–4) were also studied.

The N$_f R^+$ N$_2/O_2$ spectra of C$_{60}^+$ (z = 2–4) are shown in Fig. 7. They all include larger cluster ions, verifying that larger neutral clusters are lost from dissociating C$_{60}^+$ precursors. As the charge of the fullerene precursor increases from 2 to 3 to 4, the largest detectable neutral fragment expectedly decreases (to avoid Coulombic explosion in the remaining fragment ion) from C$_{24}$ to C$_{18}$ to C$_{12}$ [to C$_{18}$ for z = 1, cf. Fig. 3(a)]. It is worth noting that C$_1^+$ is minuscule in all spectra in Fig. 7, even for C$_{40}^+$ which would form C$_1$ with 1.1 keV kinetic energy. Thus, successive losses of C$_3$ are a minor channel; their occurrence cannot be totally excluded because the detection of such light frag-
CHARACTERIZATION OF NEUTRAL FRAGMENTS IN MS/MS

A question that is closely related to the one discussed above concerns the dissociation of \((\text{CsI})_n\) clusters. Upon CAD, these species decompose by nominal \(\text{CsI}_n\) losses, which could be intact \((\text{CsI})_n\) oligomers or a mixture of \(n\) CsI monomers (Scheme 5). The kinetic energy release distributions for the cleavage of 2 CsI units from metastable \((\text{CsI})_n\) cations, measured by El-Sayed and co-workers, were consistent with the fission of a whole \((\text{CsI})_2\) dimer rather than the consecutive loss of two CsI molecules. Reaction intermediate scans by Drewello and Vekey demonstrated that the losses of \(\text{CsI}_2\) and \(\text{CsI}_3\) from collisionally activated \((\text{CsI})_n\) cations proceed in one step as well as sequentially; whether the one-step process involves ejection of \(n\) CsI molecules or intact \((\text{CsI})_n\) multimers could not be answered. As will be shown here, neutral fragment reionization provides strong evidence for the evaporation of complete larger oligomers from energetically excited \((\text{CsI})_n\) clusters.

EVAPORATION OF OLIGOMERS FROM \((\text{CsI})_n\) CLUSTERS

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CAD of \((\text{CsI})_n\) cations with He leads to the elimination of up to three CsI units (Fig. 8). Reionization of the solvated neutrals to cations (with \(O_2\)) or anions (with Xe) gives rise to the \(+\text{CsI}^+\)/\(-\text{CsI}^-\) spectra in Fig. 9. Both of these spectra contain ions heavier than \(\text{CsI}^+\)/\(\text{CsI}^-\), indicating that oligomers (i.e. complete \((\text{CsI})_n\) moieties)
are evaporated from the collisionally activated (CsI)Cs⁺ precursors. Notice that the largest cation observed in the "Nᵢ,R⁺ spectrum [Fig. 9(a)] has three cesium atoms, whereas the largest anion observed in the "Nᵢ,R⁻ spectrum [Fig. 9(b)] has three iodine atoms. Positive and negative products emerge from the same neutral mixture. Consequently, the biggest neutral molecule in this mixture possesses three cesium and three iodine atoms and must be an intact (CsI)₁ trimer. Application of NᵢR to (CsI)₁⁺ precursors (spectra not shown) similarly reveals that they lose complete (CsI)₁ dimers during CAD. It is therefore plausible that also the loss of two CsI molecules from which gives (CsI)₂⁻ rises to the CAD base peak in Fig. 8, involves the fission of a (CsI)₂ dimer.

The only radical ions present in Fig. 9 are CsI⁺⁺ and CsI⁻⁻. Apparently, collisional ionization of (CsI)₁ and (CsI)₂ favors the formation of closed-shell fragments (CsI)₃Cs⁺ (in the positive mode) or (CsI)₁⁻ (in the negative mode). The radical ions observed presumably do not originate from reionization of the (CsI)₁ and (CsI)₂ oligomers but from reionization of CsI, which is eliminated upon CAD, too (Fig. 8). In addition, any stepwise elimination of Cs₂I₂ or Cs₃I generates CsI monomers, thereby contributing to the CsI⁺⁺ and CsI⁻⁻ peaks. The small relative abundances of the latter ions in Fig. 9 suggest, however, that the sequential losses are of lesser importance compared with the concerted evaporation of whole dimers and trimers.

Even larger oligomers are cleaved from larger cluster ions. (CsI)₅Cs⁺ and (NaI)₉Na⁺ are found to lose intact tetramers, i.e. (CsI)₄ and (NaI)₈, respectively, and the heaviest oligomer detected so far is the pentamer (CsI)₅, from (CsI)₅Cs⁺ precursor ions. The preference for elimination of intact clusters instead of a mixture of monomers could be the result of a lower energy requirement for the former process which produces thermodynamically more stable neutral molecules (see below).

Theoretical calculations by Welch et al. indicate that the most stable configurations of (CsI)₂ and (CsI)₃ have the connectivities of a rhombus and a hexagon, respectively, as depicted in Scheme 6. In these geometries, the binding energy relative to the separated monomers is 1.59 eV for (CsI)₂ and 2.69 eV for (CsI)₃. Parallel characteristics are predicted for sodium iodide dimers and trimers. Scheme 6 includes two likely structures of (CsI)₅Cs⁺ cations, as proposed by Morgan et al. and resembling a distorted cube and an elongated dodecahedron, in which alternating Cs⁺ and I⁻ form closed lattice-like surfaces. Since the faces of the latter structures have the shapes of rhombuses and/or hexagons, the evaporation of intact (CsI)₂ dimers or (CsI)₃ trimers (or even larger oligomers) could progress by the rupture of complete faces from the (CsI)₅Cs⁺ cluster. This should be true for (NaI)₉Na⁺ and other alkali metal halide cluster ions, too, all of which have been postulated to possess equivalent structures.

Collisional activation of the Na⁺ adduct of glycine, [Gly + Na⁺] (m/z 98), predominantly leads to Gly + Na⁺ (m/z 23; >50% of total fragment ion flux). The corresponding "Nᵢ,R⁺ spectrum (Fig. 10) is very similar to the CIDI spectrum of authentic glycine [Fig. 4(a)], confirming that an intact H₃NCH₂COOH molecule (75 u) is eliminated from the activated [Gly + Na⁺]⁺ precursor ion. The peak resolution is noticeably better in the "Nᵢ,R⁻ than in the reference CIDI spectrum [cf. Figs 10 and 4(a)]. This, and the small differences in the relative abundances in the two spectra, can be accounted for by the higher kinetic energy of Gly generated from [Gly + Na⁺]⁺ vs. Gly/H⁺—Gly (6.1 vs. 4.0 keV, respectively).

The copper(I) attachment ion of glycine, [Gly + Cu⁺] (m/z 138 for ⁶³Cu), also yields upon CAD the metal ion, viz. Cu⁺ (m/z 63), albeit to a smaller extent [ca. ~30% of the total fragment ion flux; Fig. 11(a)]. However, now, the corresponding "Nᵢ,R⁻ spectrum [Fig. 12(a)] indicates that the accompanying neutral loss is not an intact Gly molecule; this is attested to by the absence of a recognizable [H₃NCH₂COOH]⁺ (m/z 75) in Fig. 12(a) and the overall incompatibility of the "Nᵢ,R⁻ spectrum with the reference CIDI spectrum of pure Gly [Fig. 12(a) vs. 4(a)]. Apparently, Cu⁺ catalyzes reactions that destroy the glycine frame, in sharp contrast to Na⁺ (see below).

Another unique fragmentation of [Gly + Cu⁺] is the generation of HNCH₂Cu⁺ at m/z 92 [Fig. 11(a)], formally emerging by cleavage of a neutral with mass 46 u (H₂CO₂). Expectedly, the deuterated isotopomer [d₆-Gly + Cu⁺] loses 48 u (D₂CO₂) to produce m/z 95 (DNCΔCu⁺), cf. Fig. 11(b). Studies by Bouchonnet et al., Wen et al., and Lei and Amster have shown that the elimination of H₂CO₂ ("elements of formic acid") is a common feature of the Cu⁺ adducts of most
\[ \text{Fig. 11. CAD spectra of (a) [Gly +}^{63}\text{Cu}]^{+} \text{ (m/z 138) and (b) [d_5\text{-Gly +}^{63}\text{Cu}]^{+} \text{ (m/z 143), formed by FAB. O}_2 \text{ was used as the}\] collision gas. The narrow peak at m/z 46 (which disappears in the d_5-sample or the [^{65}\text{Cu}] adducts) is probably a matrix-related iso-
baric impurity dissociating to m/z 46 plus glycerol.

\[ \text{Fig. 12.}^{\text{+N, R}^+} \text{ spectra of (a) [Gly +}^{63}\text{Cu}]^{+} \text{ (m/z 138) and (b) [d_5\text{-Gly +}^{63}\text{Cu}]^{+} \text{ (m/z 143), formed by FAB. These spectra arise from the neutral fragments co-produced with the ionic fragments shown in}\] Fig. 11.

\[ \text{However, the}^{+N, R}^+ \text{ spectrum of [Gly + Cu]}^{+} \text{ does not contain any measurable ions at}\] m/z 46 [Fig. 12(a)]. Similarly, there is no detectable m/z 48 in the "N,R” spectrum of [d_5\text{-Gly +}^{63}\text{Cu}]^{+}, cf. Fig. 12(b). Hence neither formic acid nor dihydroxycarbene is eliminated.

\[ \text{Regarding the alternative}\] H_2CO_3 neutral losses, viz. H_2 + CO_2 and H_2O + CO, characteristic "N,R” ions are observed only for their heavier components; these appear in Fig. 12(a) at m/z 44 (CO_2”) and 28 (CO”). Their presence is substantiated by the "N,R” spectrum of [d_5\text{-Gly +}^{63}\text{Cu}]^{+} [Fig. 12(b)] which includes clearly resolved peaks for CO_2” (m/z 44) and CO” (m/z 28). The lighter constituents of the above H_2CO_2 (D_2CO_2) neutral products, viz. H_2(D_2) and H_2O (D_2O), are discriminated against by more severe scattering losses and poorer reionization and transmission efficiencies (see above), which obstruct their detection and can thus explain their negligible contribution to the "N,R” spectra (water gives just a trace signal). With consideration of this limitation, it is concluded that the neutral fragment of mass 46 \text{ u} is eliminated in the form of H_2 + CO_2 and/or H_2O + CO.

\[ \text{Being a relatively soft Lewis acid, Cu}^+ \text{ presumably attaches close to the soft amino group of glycine (a in}\] Scheme 7), additional coordination by the carboxyl substituent is plausible.\[ \text{Scheme 7.}\]

A hint as to what happens to this structure when collisionally activated is given by the neutral fragments that can be identified by "N,R”. The heaviest organic (i.e. non-copper containing) neutral lost from [Gly + Cu]^{+} is the carboxyl radical, COOH (45 u), which after reionization gives rise to a recognizable signal at m/z 45 [Fig. 12(a)]; similarly, [d_5\text{-Gly +}^{63}\text{Cu}]^{+} cleaves COOH (46 u, cf. Fig. 12(b)]. The residual fragment of glycine, viz. H_2NCH_2O: of 30 u (or D_2NCH_2O: of 34 u) is also present in the neutral loss mixture, as attested by the observation of m/z 30 (or 34) in Fig. 12(a) (or 12(b)). These results strongly suggest that copper(I) catalyzes the cleavage of the C–COOH bond, possibly by insertion, as shown in Scheme 7.56–58

In the resulting intermediate b, the glycine connectivity is destroyed, consistent with the absence of intact Gly molecules in the neutral fragment mixture from [Gly + Cu]^{+} (see above). Significant CAD fragment ions that can be rationalized by direct cleavages from b are (i) "H_2NCH_2 (m/z 30 in Fig. 11(a)), (ii) Cu" (m/z 63) and (iii) "CuCOOH (m/z 108). The complementary neutral losses are the aforementioned radicals (i.e. COOH, H_2NCH_2, and Cu (63 u), all of which are detected in the "N,R” spectrum [Fig. 12(a)]. Further, as has been proposed by many other researchers,56–58 simple hydrogen or hydroxyl rearrangements in b can account for the other products observed in CAD and "N,R” spectra, inter alia HN=CH_2 – Cu" (m/z 92), loss of H_2O + CO or H_3 + CO_2 and CuOH_2^+ (m/z 81; loss of HN=CH_2 + CO). The heaviest neutral fragment observed is CuOH (80 u)/CuOD (81 u), cf. Fig. 12. Such a molecule can be envisioned as arising from b.

\[ \text{x-amino acids. Several different molecules are possible as the neutral fragment(s) in this process, including HCOOH (formic acid), CO}_2, \text{H}_2\text{O + CO or a combination thereof. Here, this uncertainty is partially resolved by N}\] reionization of this limitation, it is concluded that the neutral fragment of mass 46 \text{ u} is eliminated in the form of H_2 + CO_2 and/or H_2O + CO.

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by OH\(^{-}\) transfer to Cu\(^{+}\) and cogeneration of \(\ce{H2N=CH2+CO}\). Reionization of CuOH/CuOD gives rise to CuOH\(^{+}\)/CuOD\(^{+}\) and CuO\(^{+}\) (m/z 79) and presumably also is a partial source of Cu\(^{+}\) (m/z 63) in the \(^{+}N_{1}R^{+}\) spectra.

Competitive pathways that disrupt the glycine structure are conceivable. For example, transition metal ions are known to increase the acidity of amines in solution.\(^{62}\) Proton transfer from the amino to the carboxyl group of Gly could then be facile, yielding the ion–molecule complex c as depicted in Scheme 8. Some important CAD fragments that can be formed from c by ligand evaporation are \(\ce{HN=CH2+C=O}^{+}\) (m/z 92) and CuOH\(^{+}\) at (m/z 81) + CO + HN=CH\(_2\); other products, such as \(^{+}H_{2}N=CH_{2}\) (m/z 30) + CO + CuOH, could emerge via proton transfer reactions within c.

\[
\text{Scheme 8.}
\]

Finally, the m/z 42 ion in the \(^{+}N_{1}R^{+}\) spectrum of [Gly + Cu]\(^{+}\) is probably \(\ce{CH_{3}==C=O}^{+}\) (ionized ketene). With \(\text{d}_{5}-\text{Gly + Cu}]^{+}\), this ion shifts to m/z 44 and overlaps with CO\(^{+}\) (Fig. 12). Ketene loss suggests that not all fragmentations of [Gly + Cu]\(^{+}\) cleave the C=COOH bond. CH\(_{3}\)=C=O could be eliminated, together with H\(_{2}\)N\(^{-}\) or ‘OH, upon generation of CuOH\(^{+}\) (m/z 80) or CuNH\(_{2}\)\(^{+}\) (m/z 79), respectively.

Alanine completely parallels glycine in its reactivity towards Na\(^{+}\) vs. Cu\(^{+}\). Thus, CAD of [Ala + Na\(^{+}\)] mainly yields Na\(^{+}\) by liberation of a whole alanine molecule; this is confirmed by the striking similarity of the \(^{+}N_{1}R^{+}\) spectrum of [Ala + Na\(^{+}\)] (Fig. 13) with the reference CIDI spectrum of authentic Ala [Fig. 4(b)]. In contrast, collisionally activated [Ala + Cu\(^{+}\)] does not eliminate an intact alanine unit upon dissociation to give Cu\(^{+}\). In analogy with [Gly + Cu\(^{+}\)], the copper(I) adduct of Ala shows an abundant loss of H\(_{2}\)CO\(_{2}\) (46 u) in its CAD spectrum and significant H\(_{2}\)O, CO, CO\(_{2}\) as well as CuOH losses in its \(^{+}N_{1}R^{+}\) spectrum. Taking the dissociation behavior of [Gly + Cu\(^{+}\)] (or [Ala + Cu\(^{+}\)]) in reverse, copper(I) complexed with appropriate ligands (e.g. an imine, water and carbon monoxide) may catalyze reactions among the ligands that ultimately produce an amino acid. This hypothesis could be tested by studying the ion–molecule reactions between Cu\(^{+}\) and suitable molecules.\(^{63}\)

**STRUCTURAL INFORMATION FROM NEUTRAL LOSSES**

Certain isomeric ions show only minor differences in their CAD spectra, because they mainly decompose to common fragment ions by elimination of different neutrals.\(^{64}\) This is true for the [M + H\(^{+}\)] cations from isomeric mononucleotides, whose major CAD channel yields the protonated nucleobase by elimination of different sugar residues, as exemplified for the cytidine-monophosphates in Scheme 9.\(^{65}\) In this and in related instances, characterization of the neutral CAD fragments may provide useful structural insight.

![Figure 13. \(^{+}N_{1}R^{+}\) He/O\(_{2}\) spectrum of [Ala + Na\(^{+}\)], formed by FAB at 8.0 keV. This spectrum is strikingly similar to the CIDI spectrum of 4.0 keV Ala depicted in Fig. 4(b). Note that Ala released from [Ala + Na\(^{+}\)] has 4.7 keV kinetic energy.](image-url)
The major neutral losses from these precursor ions are monophosphates, formed by FAB. (a) 2'~CMP, (b) 3'~CMP and (c) 5'~CMP. The major neutral losses from these precursor ions are (a) 1, (b) 2 and (c) 3 (cf. Scheme 8).

Figure 14. *N,R*+ He/O2 of [M + H]+ from isomeric cytidine-monophosphates, formed by FAB. (a) 2'~CMP, (b) 3'~CMP and (c) 5'~CMP. The major neutral losses from these precursor ions are (a) 1, (b) 2 and (c) 3 (cf. Scheme 8).

shown in Scheme 10. Note that there is a significant difference between 1 and 2 in the abundance ratio [m/z 122]:[m/z 123], which is >1 for 1 but <1 for 2 (appraised by overlaying the spectra). A stable cyclic m/z 122 (Scheme 10) can arise via simple bond cleavages only from 1, justifying the uniqueness of this product for the vicinal phosphate 1. Compared with 1 and 2, isomer 3 from [5'~CMP]H+ bears no hydroxymethyl substituent and yields no m/z 181 or any of the fragments proposed to result from m/z 181 (Scheme 10). The absence of higher mass ions in the reionization spectrum of 3 suggests that this neutral suffers more extensive fragmentation upon collisional ionization (CIDI), possibly owing to increased ring strain.

Finally, the ion of m/z 111 appears with higher relative abundance in the spectrum of [5'~CMP]H+ than in the other two spectra Fig. 14(c) vs. Fig. 14(a) and (b). This "N,R"+ product corresponds to the molecular ion of the nucleobase (cytosine), which is also eliminated to a small extent from the collisionally excited [M + H]+ precursor ions (<10% of the major channel which is formation of the protonated nucleobase by loss of 1~3, cf. Scheme 9). The reason for the higher abundance of m/z 111 from [5'~CMP]H+ is not known.

Based on the data discussed, the most useful structural information is provided by the principal neutral losses 1~3; the neutral product from [5'~CMP]H+, i.e. 3, is readily distinguished from its isomers 1 and 2. On the other hand, 1 and 2 behave more similarly upon reionization but can still be differentiated using the m/z 122 product which is diagnostic for 1 (see above). Overall, the knowledge gained by N,R, combined with the small differences observed in the CAD spectra, can help assign the correct nucleotide structure with increased confidence.

OUTLOOK

Neutral fragment reionization makes it possible to 'see' what neutral species are eliminated from activated precursor ions. Characterization of these fragments supplies information that is essential in establishing correct decomposition mechanisms, as has been demonstrated here for a variety of systems. Understanding dissociation mechanisms both aids spectral interpretation and structural assignments and gives clues on how the same ions may be formed by association of smaller moieties. Neutral fragment identification also provides structural information on the dissociating precursor ion; the latter aspect is particularly important when structural characterization based on ionic fragments, which is the traditional MS/MS approach, is ambiguous or difficult.

These capabilities are currently accompanied by a few weaknesses. Specifically, (i) collisional reionization has a low yield (see Experimental) and produces many fragments from each neutral loss. For certain neutral species, it mainly leads to low-mass products and weak, if any, molecular ions, as is the case with amino acids (Fig. 4) and the glycophosphates 1~3 (Fig. 14). (ii) The product ions from different neutrals may overlap, thereby complicating spectral interpretation. (iii) Light neutral losses are more difficult to detect than equivalent amounts of heavier neutrals. Generally, the larger neutral fragments dominate N,R spectra, because their higher kinetic energies equip them with superior reionization cross-sections and transmission and collection efficiencies. (iv) The spectral resolution is limited if the reionization products are separated by an electrostatic analyzer (ESA), as has so far been the case in the most CIDI and N,R studies.

The above deficiencies can adequately be overcome by a softer reionization method and instrumental modifications. A technique that forms one ion per neutral (preferably the molecular ion) would permit the identification of the neutral fragments directly from their mass-to-charge ratios. Chemical reionization is an appropriate alternative. It requires that the neutrals move slowly and would, therefore, be ideally suitable for tandem quadrupole mass spectrometers; also, such
instruments would not discriminate against light neutrals. With sector instruments, chemical reionization could be achieved by decelerating the precursor ions before dissociation, so that they yield slow neutral fragments. Resonance-enhanced multiphoton ionization represents another soft reionization method and might be useful for the selective and sensitive detection of a specific neutral fragment; with pulsed lasers, this method would require phase-sensitive or array detection.

The other major obstacle in N_R studies, namely the poor resolution, can be alleviated by linked scanning if a further mass-analyzing device, for example a quadrupole, is attached to the simple electrostatic analyzer currently used for N_R product separation.

Satisfactory answers to these problems would both extend the applicability of neutral fragment reionization to larger precursor ions and also substantially simplify the interpretation of N_R data. The promise of N_R relies on the fact that there is a vast array of precursor ions (besides those presented here and in earlier reviews) that await direct characterization of their neutral fragments, because they either decompose via mechanisms not yet clarified or yield an insufficient number of ionic fragments for complete structure or sequence diagnosis. Current studies in our laboratory are aimed at establishing whether protonated peptides can lose their N-termini as oxazolones; this important question has arisen after the N-terminal sequence ions were recently shown to be protonated oxazolones. Other systems worth investigating are sodiated oligopeptides (they undergo puzzling eliminations at the C-terminal amino acids), lithiated saccharides (their neutral fragments remain unknown), peptide complexes with transition metal ions (they often produce incomplete sequence ion series) and various types of cluster ions (to elucidate on their evaporation mechanisms).

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